Title: RESISTANCE TO VERTICILLIUM 2 IN TOMATO

Abstract: A cultivated type tomato plant having a high level Verticillium race 2 resistance.
RESISTANCE TO VERTICILLIUM RACE 2 IN TOMATO

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The invention relates to the field of plant breeding, and more particularly, to the development of tomato varieties and lines that are resistant to Verticillium wilt caused by the fungus Verticillium dahliae Race 2.

Description of Related Art

[0002] Tomatoes are one of the most important vegetable crops in many countries, including countries of Europe, the Americas and the Asian Pacific countries. Tomatoes are a significant cash crop in the United States of America. The California processing tomato crop accounts for over 95% of U.S. production, with farm gate value in excess of $500 million annually and retail product value of over $4 billion.

[0003] Tomato production all over the world is affected and limited by Verticillium wilt, caused by the fungus Verticillium dahliae. The fungus enters tomato roots and causes brownish discoloration and then develops into the stems and leaves. The most typical symptom is wilting of the leaves with wilt symptoms developing initially at the bottom branches and progressively ascending until it reaches the top of the plant. The disease causes significant economic loss in many growing regions. Yield reductions of 25% to 69% have been observed on susceptible cultivars (Jones and Crill, 1975, Pl. Dis. Rep.).

[0004] Effective control of Verticillium wilt disease has been found by planting resistant cultivars. In 1951 resistance to the initial race of Verticillium wilt was found in a red small-fruited wild species L. esculentum var. cerasiforme. This resistance, controlled by a single dominant gene (Ve), has been incorporated into many current tomato varieties (Schaible et al., 1951, Phyt.)

[0005] The resistance to this initial Verticillium wilt race, now designated as race 1, was broken in 1957, when new Verticillium isolates from California and Canada were reported to be pathogenic to a tomato variety carrying the Ve gene. These new isolates, able to attack cultivars with the Ve gene, were designated race 2. Race 2 has now been identified in many countries, and is well established throughout the world: Canada (Robinson et al., 1957, U. Wiss. B.); Brazil (Laterrot et al., 1983, Hort. Bras.); Chile (de Badilla, Agr. Tec., Santiago); France (Laterrot, 1968); Italy (Cirulli, 1969, Phytop. Mediterr.); Spain (Baergen & Hewitt, 1988, Hort. Sci.); Greece (Tjamos, 1980, Phytop.); Crete (Lygoxigakis & Valakounakis, 1992, Pl. Pathol.); Romano (Costache & Tomesku, 1987, Bull. D'Acad. Sci.
Rom.; Australia (O'Brien & Hutton, 1981, Aust. Pl. Path.); Morocco (Besri et al., 1984, Phyt. Z.); South Africa (Ferreira et al., 1990, Pl. Dis.).

[0006] In 1975 the incidence of race 2 was discovered to be 100% in 46 surveyed field in California (Grogan et al., 1979, Phytop.). In 1980, race 2 appeared in two-third of the tomato fields in North Carolina (Okie & Gardner, 1982, Hort. Sci.).

[0007] Race 2 continues to gain territories, at least somewhat expected, as farmers continue to grow hybrids resistant to race 1 (Bender & Shoemaker, 1984, Pl. Dis.).

[0008] Race 2 is an increasing economic problem. Grogan et al. (1979, Phytop.) reported a 25% reduction in yield for processing tomatoes grown in California. The same reduction in yield is reported for North Carolina and Canada (Bender & Shoemaker, 1984, Pl. Dis.; Tabaeizadeh et al., 1997, Pl. Phys.). It is widely accepted in fact, that Verticillium race 2 and Corky root represent the two diseases causing the most significant losses to California tomato farmers (Ch. Rivara, Tomato Dis. Worksh., 2002).

[0009] The search for resistance to Verticillium race 2 has been going on for more than two decades. The growing prevalence of race 2 and the yield reduction has attracted the attention of tomato researchers and breeders in U.S.A. and Europe. A short review of the tomato industry's need, intense research activity, for finding a reliable source for resistance to race 2 is demonstrated by the following papers, with their highlighted conclusions:


[0011] 1979 - R.G. Grogan et al., in "Verticillium on Resistant Tomato Cultivars in California: Virulence of Isolates from Plant and Soil... (Phytopathology, Vol. 69, No 11, 1176-1179): "The effort to find a reliable source of resistance to race 2 must be continued and intensified"


After testing 141 tomato lines from Plant Gene Resources of Canada, the USDA Regional Plant Introduction Station and from seed companies, the authors found line MEL to have moderate resistance, however, to be controlled by 3 recessive genes, making this resistance almost useless to breeders, due to the very complex breeding required to introgress 3 separate genes into a commercial variety. That is the reason the MEL source has not been used in any tomato breeding program, even though it was reported more than 20 years ago.

1984 - H. Laterrot (Tomato Genetics Cooperative Report) in France showed that MEL line was susceptible to some isolates of race 2 from Brazil. He found a line from Martinique to be less susceptible to attack by race 2. This line has been tested by some seed companies in the U.S., but the level of resistance was not high enough for use in breeding.

1984 - C.G. Bender and P.B. Shoemaker in "Prevalence of Verticillium Wilt of Tomato and Virulence of Verticillium dahliae Race 1 and Race 2 isolates in Western North Carolina" (Plant Disease/April, 305-309): "UNfortunately, NO SOURCE OF HIGH LEVEL OF RESISTANCE TO RACE 2 HAS BEEN FOUND" (Also: D.H. Hall and K.A. Kimble, 1972, California Agriculture, 26(9): 3)

1993 - K.D. Baergen, J.D. Hewitt and D.A. St. Clair in "Resistance of Tomato Genotypes to Four Isolates of Verticillium dahliae Race 2" (Hort. Science, 28(8):833-836): "Durable genetic resistance to race 2...is particularly desirable as a control method"..."NO TOMATO GENOTYPE DEMONSTRATED RESISTANCE TO ANY OF THE RACE 2 ISOLATES".

The authors tested all lines previously reported as resistant genotypes and found that they "did not fall into discrete categories of resistant and susceptible, rather within a continuous range of greater or lesser susceptibility."

1998 - T. Gordon (CTRI Annual Report) after screening many wild species reported some resistance in the small-fruited wild species L. pimpinellifolium LA 722. This accession showed susceptibility to an aggressive California race 2 isolate Vd 9701 and it has not been used in tomato breeding programs.

2003 - J. Stommel in his report "An integrated Approach for Control of Verticillium dahliae Race 2 in Tomato", presented at the Tomato Breeders RoundTable (Utah): "TOMATO CULTIVARS RESISTANT TO RACE 2 ARE NOT AVAILABLE". After testing 212 accessions of the USDA, ARS wild species Lycopersicon pimpinellifolium collection in Beltsville, MD, the researcher failed to discover a suitable level of resistance to race 2.

2004 - Ch. Rivara, Director California Tomato Research Institute, in "Public and Private Partnerships: the Evolving California Experience", a report, presented at the
Worldwide Congress for Processing Tomato, November, Melbourne, Australia: "Areas of interest now include...screening for WIDELY NEEDED SOURCE OF RESISTANCE TO VERTICILLIUM WILT RACE 2 (V. dahliae)..."

[0022] 2005 - Ch. Rivara in "California Processing Tomatoes - News and Information": "The ENTIRE TOMATO INDUSTRY HAS BEEN SEEKING A SOURCE OF RESISTANCE FOR VERTICILLIUM WILT (V. dahliae) RACE 2 SINCE IT'S DISCOVERY IN EARLY 1970's...The current CTRI project uses resistance line developed by Dr. Liliana Stamova...Her studies demonstrated a high level of resistance..."

[0023] The above review shows that even though race 2 was detected as early as 1957, the industry continues to struggle to develop cultivars with a suitable level of resistance to race 2 of Verticillium dahliae. During the past several decades of screenings of various germplasms no natural source with a reliably high level of resistance has been developed to be used in the tomato breeding programs. To date, no resistant cultivars are available to tomato growers in U.S. and other countries, and there are no lines available to breeders of any type or level of resistance having a convenient or simple mode of inheritance.

[0024] Therefore, there remains a need for a tomato source with a high level of resistance to race 2 of Verticillium dahliae.

**SUMMARY OF THE INVENTION**

[0025] The invention provides a cultivated type tomato plant, such as a Lycopersicon esculentum plant, having a high level Verticillium race 2 resistance. The invention further provides a Lycopersicon esculentum tomato plant having resistance to both Verticillium race 2 and Verticillium race 1. In a preferred such embodiment the tomato plant has the Ve gene. The trait for high level Verticillium race 2 resistance is preferably dominantly inherited.

[0026] In one embodiment, the tomato plant is homozygous for the Verticillium race 2 resistance trait.

[0027] In one preferred embodiment, the high level Verticillium race 2 resistance is derived from a tomato plant of the species Lycopersicon chilense Dun.

[0028] The invention also provides seed, pollen, ovules, and other vegetative tissues from such plant, or a tomato plant regenerated from such tissue.

[0029] The invention also provides a method for producing hybrid tomato seed comprising crossing an inbred tomato plant having a high level Verticillium race 2 resistance with a second tomato plant and harvesting resultant hybrid tomato seed.

[0030] Thus, the invention provides a hybrid tomato plant produced by growing the resultant hybrid tomato seed.

[0031] A method for producing a high level Verticillium race 2 resistance tomato
plant is also provide, by crossing a female cultivated tomato plant with a male tomato plant of
the subgenus *Eriopersicon* having race 2 resistance. In one embodiment the male tomato
plant is *Lycopersicon chilense* Dun., and in another embodiment the female cultivated tomato
plant is *Lycopersicon esculentum* Mill.

[0032] The tomato plant of the invention preferably produces a fruit having a
diameter of at least about 5 cm.

[0033] The invention also provides tomato fruit harvested from a high level
*Verticillium* race 2 resistance tomato plant and tomato seed derived from such tomato fruit.

[0034] The invention also provides a plurality of tomato plants with high level
*Verticillium* race 2 resistance grown in a field.

[0035] In another embodiment, the tomato plant has the high level *Verticillium* race
2 resistance characteristic of a plant produced from seed deposited as Accession No. NCIMB
41327. In one preferred embodiment, the plant has resistance to both *Verticillium* race 2 and
*Verticillium* race 1

[0036] In another preferred embodiment, the tomato plant is descended from a plant
first produced as seed deposited as Accession No. NCIMB 41327. Such a plant will, in one
such embodiment, have resistance to *Verticillium* race 1.

[0037] The invention also provides seed, pollen, ovules, and other vegetative tissues
from such descendent plant, or a tomato plant regenerated from such tissue.

[0038] The invention also provides a method for producing hybrid tomato seed by
crossing an inbred descendent tomato plant with a second tomato plant and harvesting the
resultant hybrid tomato seed.

[0039] The invention further provides hybrid tomato plants produced by growing the
resultant hybrid tomato seed.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[0040] Various exemplary embodiments of this invention will be described in detail,
with reference to the following figures, wherein:

[0041] Figure 1 is a photographic depiction of *Verticillium* wilt symptoms in a
susceptible control (right) compared to the symptom free appearance of the VEDA line (left
and center).when each is challenged with *Verticillium dahliae*.

[0042] Figure 2 shows tomato fruit of the resistant VEDA line.

[0043] Figure 3 is a photographic depiction of *Verticillium* wilt symptoms in
susceptible control plants compared to the symptom free appearance of VEDA line plants
challenged by *Verticillium dahliae* isolate TSA-S-5.

[0044] Figure 4 is a photographic depiction of VEDA line and susceptible control
two months after the insulation with isolate TSA-S-5. ADD

[0045] Figure 5 is a photographic depiction of VEDA line and susceptible control after the inoculation with isolate TSA-S-5. ADD

DESCRIPTION OF THE PREFERRED EMBODIMENT

[0046] For purposes of this application, the absence of symptoms at the two month level after the inoculation protocol using *Verticillium dahliae* isolates as described herein, indicates a "high level *Verticillium* race 2 resistance". Additionally, for lines having high level *Verticillium* race 2 resistance, fewer than 3% of the plants will typically show any wilting symptoms at all beyond the first set of true leaves when challenged under the inoculation protocol.

[0047] "Wilting symptoms" or "wilt" refers the symptoms of *Verticillium* race 2 infection in susceptible plants, including chlorosis and wilting of leaves, typically developing at the bottom leaves and ascending progressively until the symptoms reaches the top of the plant. Susceptible plants will show substantial wilting symptoms over 50% or greater of the plant at 60 days, when challenged under the inoculation protocol *infra*.

[0048] Reference to an element by the indefinite article "a" or "an" does not exclude the possibility that more than one of the element is present, unless the context clearly requires that there be one and only one of the elements. The indefinite article "a" or "an" thus usually means "at least one".

[0049] The term "comprising" is to be interpreted as specifying the presence of the stated parts, steps or components, but does not exclude the presence of one or more additional parts, steps or components. A plant comprising a certain trait may thus comprise additional traits.

[0050] Whenever reference to "plants" is made, it is understood to also include plant parts (cells, tissues, seeds, severed parts such as heads and/or stalks), progeny of the plants which retain the distinguishing characteristics of the parents (especially the raised head and leafless stalk), such as seed obtained by selfing or crossing, e.g. hybrid seed (obtained by crossing two inbred parental lines), hybrid plants and plant parts derived therefrom are encompassed herein, unless otherwise indicated.

[0051] The genus *Lycopersicon* is divided into two subgenera, *Eulycopersicon* and *Eriopersicon*. The former includes the red-, orange-, or yellow-fruited species *L. esculentum*, *L. cheesmanii*, and *L. pimpinellifolium*. *Eriopersicon* comprises the green-fruited species *L. chilense* Dun., *L. chmielewskii*, *L. hirsutum*, *L. parviflorum*, *L. pennelli*, and *L. peruvianum*.

[0052] The name "VEDA" is given to the high level *Verticillium* race 2 resistant *L. esculentum* tomato lines described herein, derived from an introgression from the
*Eriopersicon* species *L. chilense* Dun. The VEDA line is presently the only line available showing the high level of resistance to race 2 of *Verticillium dahliae* necessary for use in seed tomato breeding programs.

**0053** Various inbred tomato plants may be developed from the VEDA line for commercial purposes by known breeding techniques of crossing and selection, for agronomic traits such as disease resistance, regional, soil and climate adaptation, and maturity, as well as quality traits commonly bred for tomato, including fruit shape and size, color, flavor, splitting and ripening. The term “inbred tomato plant” also includes any single gene conversions of that inbred. The term “single gene converted plant” as used herein refers to those tomato plants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the single gene transferred from the donor parent into the inbred via the backcrossing technique.

**0054** Also provided is a method of producing hybrid tomato plants having a high level *Verticillium* race 2 resistance. This method involves crossing two (preferably inbred) plants according to the invention and harvesting the hybrid seeds. As the VEDA line has been shown to be dominant, uniform and stable for all traits, F1 seeds obtained will, when grown, have *Verticillium* race 2 resistance, as defined.

**0055** The development of commercial tomato hybrids involves the development of homozygous inbred parental lines through techniques well known to the art. Generally, two or more germplasm sources or gene pools are combined to develop superior hybrid plants. Desirable inbred or parent lines are developed by continuous selection, followed up with several generations of selfing until the lines are sufficiently uniform. Alternatively, anther or microspore culture (followed by chromosome doubling to produce double haploids lines, also referred to as “DH” lines) may be used followed by selection of the best breeding lines and testing progeny in various hybrid combinations.

**0056** Once the inbred lines that give the best hybrid performance have been identified, hybrid seed can be produced indefinitely, as long as the homogeneity and the homozygosity of the inbred parents is maintained. The commercial hybrid seed is produced in the open field or in the greenhouse, using methods well known to the art.

**0057** This invention also is directed to methods for producing a tomato plant by crossing a first parent tomato plant with a second parent tomato plant wherein either the first or second parent tomato plant is an inbred tomato plant of the VEDA line. Further, both first and second parent tomato plants can come from the inbred tomato VEDA line. Still further, this invention also is directed to methods for producing an inbred tomato VEDA line-derived tomato plant by crossing inbred tomato VEDA line with a second tomato plant and growing
the progeny seed, and repeating the crossing and growing steps with the inbred tomato \textit{VEDA} line-derived plant from 0 to 7 times. Thus, any such methods using the inbred tomato \textit{VEDA} line are part of this invention: selfing, backcrosses, hybrid production, crosses to populations, and the like. All plants produced using inbred tomato \textit{VEDA} line as a parent are within the scope of this invention, including plants derived from inbred tomato \textit{VEDA} line. Advantageously, the inbred tomato line is used in crosses with other, different, tomato inbreds to produce first generation (F1) tomato hybrid seeds and plants with superior characteristics.

[0058] Traditionally, the hybridization of tomato plants is done by manual emasculation and pollination. This is a labor-intensive process and does not guarantee 100\% hybridity. In many other crop varieties male-sterile systems exist which have been genetically engineered into plants for more efficient production of hybrid varieties, and such male-sterile systems are increasingly available in tomato. Thus, it should be understood that the inbred can, through routine manipulation of cytoplasmic or other factors, be produced in a male-sterile form. Such embodiments are also contemplated within the scope of the present claims.

[0059] With the \textit{VEDA} line resistant to race 2, it will be very easy for breeders to develop tomato lines, varieties and hybrids combining resistance to both race 1 and race 2, as they will only need to introgress the resistance to race 2 from one of the parents (the resistance in \textit{VEDA} is dominant).

[0060] The availability of hybrids with resistance to Verticillium wilt derived from two different wild species will allow breeders, from one side, to augment the level of resistance and, from the other side, to slow the evolution of the pathogen by opposing to it a combination of different factors for resistance.

[0061] \textit{Verticillium dahliae} infects many plant species. Host plants include vegetables, field crops, ornamentals, trees, some grasses and weeds - cotton, mint, safflower, strawberries, potato, cantaloupe, apricot, peach, olive... (C.G. Bender and P.B. Shoemaker, in Plant Disease, 1984; E.C.Tjamos, Phytopathology, 1981). Tomatoes are part of crop rotation in the field and having healthy tomato crop will help to avoid infection in other crops, like pepper, eggplant, strawberries, cotton.

[0062] The following examples are intended to illustrate but not to limit the invention.

\textbf{Example 1. Description Of The Source Materials And Procedure Of Developing VEDA Line}

[0063] The original interspecific cross was made by crossing a local Bulgarian cultivar of \textit{Lycopersicon esculentum}, as female parent, to the wild tomato species \textit{L. chilense} Dun. The local cultivar is a determinate plant.

[0064] The fruits of the interspecific cross were large, round, and uniform green.
Two crosses to the cultivated tomato were made, followed by six generation of self-pollinations and selection.

[0065] Screening for resistance to race 2 was performed in a plastic greenhouse. Two-week old seedlings were inoculated by using the standard root-dip technique. Disease symptoms were evaluated weekly for a period of two months. The evaluation for resistance to race 2 was based mainly on the severity of the foliar symptoms - chlorosis and wilting, and the discoloration of the lower part of the stem, as well as some checking for fungus presence into the stem.

[0066] All selections for horticultural traits were made in the field. Selection was applied for the following traits: indeterminate habit of the plants, size and shape of the fruits, uniform green fruits, intensive red color in mature stage, good fruit setting and earliness.

Example 2. Description Of The Wild Tomato Species L. Chilense Dun. Participating In The Development Of VEDA Line

[0067] L. chilense Dun (Subgenus Eriopersicon) is an wild tomato species originated from the arid areas of South Peru/North Chile. The fruits are very small (1.2-1.4 cm diameter), gray green, never becoming red at the mature stage. The stem is covered by short, thick hair.

[0068] The cultivated tomato (L. esculentum Mill) does not cross easily with the wild species from Subspecies Eriopersicon (green fruited tomatoes). In the case with L. chilense (with the same chromosome number as tomato, 2n = 2x = 24) tomatoes are crossed more easily if the cultivated variety is used as a female parent. Backcrosses (BC) with the cultivated tomato are also more easily achieved if the tomato is the mother parent. The fertility in BC1 provides opportunity for using L. chilense in tomato breeding programs.

Example 3. Description Of VEDA Line

[0069] The developed VEDA line is a cultivated type tomato. The plants are indeterminate (three or more leaves between the inflorescences), very vigorous and with strong root system.

[0070] The VEDA line is relatively late in maturity. It has a good fruit setting when grown in the field. The inflorescences are simple, bearing 5 - 6 fruits per cluster. The fruits are mid-size (50-60 g), red, round, and uniform green. Figure 2.

Example 4. Greenhouse Tests With VEDA Line

[0071] Greenhouse tests with VEDA line have been performed for several years in California. The plants were inoculated separately with two race 2 isolates - TSA-S5 (the widely spread strain in California) and Vd 9701 (a virulent strain), both obtained by the Dept. of Plant Pathology at the University of California, Davis.

[0072] A standardized inoculation protocol was followed in all experiments. Roots
or young plants (two well developed true leaves) are washed in tap water, trimmed to 2.5 cm in length and immersed for 10 min in a spore suspension. The concentration is adjusted to $10^5 - 10^6$ spores/ml. Control plants are dipped into tap water. Inoculation plants are then transplanted into pots and arranged randomly into two blocks per isolate.

[0073] Disease reactions are evaluated weekly for 8 weeks base on foliar symptoms - wilting and chlorosis of cotyledons and leaves. The severity of symptoms is estimated using a scale of 0 - 4, where 0 = no symptoms, 1 = chlorosis and wilting of cotyledons, 2 = chlorosis and wilting of first or second true leaves, 3 = symptoms on lower 50 % of the foliage, 4 = symptoms on 51 - 100 % of the foliage. Plants with ratings 0, 1 and 2 are considered resistant, i.e., plants showing no chlorosis or wilting above the level of the first or second true leaves.

[0074] VEDA plants show no symptoms at all, or, with some plants only, the cotyledons and the first and second leaves showed mild wilting. Whenever present, the wilting on VEDA lines started normally later than the symptoms on the susceptible control plants, and they do not go up or ascend in the plant as the plant matures. At the same time the susceptible control plants show intensive chlorosis, typical V-shaped lesions and wilting that go up and continue throughout the plant.

[0075] The differences in response of VEDA line and the susceptible control were more obvious two months after the inoculation, at the time of fruit setting. The symptoms became even more profound 4 - 5 months after the inoculation. The susceptible control is heavily damaged as Verticillium wilt symptoms reach the very top of the plants and the lower part of the stems show dark brown discoloration. At the same time VEDA plants have vigorous green leaves. Figure 3 shows a VEDA plant to the right, with vigorous growth, and a susceptible plant to the left, having chlorosis and wilt resulting in substantial loss of leaf cover across the entire plant.

Example 5. Field Trial With VEDA Line

[0076] A field trial with VEDA line was carried out in 2002 at the University of California in Davis. The plot used for screening was artificially inoculated for several years with two isolates, including the virulent Vd 9701 strain. Additional inoculum was applied at the time of transplanting. The inoculum consisted again of a mixture of two race 2 isolates.

[0077] The VEDA line showed strong resistance in the field - no foliar symptoms, no vascular discoloration were detected. Most of the samples, taken from the lower part of the stems of VEDA plants and investigated in the laboratory, appeared to be free of Verticillium.

[0078] At the same time the nearby susceptible control plants showed typical Verticillium wilting to the top of the plants and pronounced discoloration into the stem, where
verticillium presence was detected. These field trials established the high levels of resistance in the VEDA lines, compared to the complete wilt symptoms and significant yield loss in the control susceptible lines.

**Example 6. Challenging VEDA Line With Different Isolates Of Verticillium Race 2 From California**

**0079** In 2003 the VEDA line was inoculated separately with 10 isolates of race 2 from different tomato growing regions in California. The isolates were provided by Dr. Mike Davis (UC, Davis) and by five seed companies.

**0080** VEDA line showed high level of resistance to all isolates.

**Example 7. Genetics Of Resistance To Verticillium Race 2 In VEDA Line**

**0081** To study the genetic of resistance to race 2 in VEDA line the responses of the following lines and populations were evaluated:

**0082** Lines: VEDA = P1 parent, 155 (Ve/Ve, resistant to race 1 and susceptible to race 2) = P2 parent, Glamour - susceptible control (without Ve gene).

**0083** Populations: F1 (VEDA x 155), F2 (VEDA x 155), Backcrosses BC1P1 and BC1P2. Line 155 VFFNPto is a property line belonging to the California Tomato Research Institute (CTRI).

**0084** Two weeks old plants were inoculated separately with two isolates - TSA-S5 (the widely spread in California strain) and the virulent strain Vd 9701. The method of inoculation and the evaluation of the disease severity were the same as described for the greenhouse tests with VEDA line.

**0085** The level of resistance of F1 plants was comparable to that of VEDA line (Table 1). One plant rated 3 might be explained with the interaction between the pathogen, host and the environment that might cause appearance of phenotypes that do not represent their genotypes (Okie and Gardner, 1982).

**0086** The expression of resistance in BC1P1 (backcross to VEDA) was the same as for the VEDA line.
TABLE 1. Reaction of lines, F1, F2, BC1P1 and BC1P2 to isolate TSA-S5 of *Verticillium dahliae* race 2 at 60 days after inoculation.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Disease Severity</th>
<th>Total Plants (No.)</th>
<th>Expected Ratio R:S</th>
<th>$\chi^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0    1    2    3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>VEDA</strong> = P1</td>
<td>25   3    2    0</td>
<td>30</td>
<td>1:0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>155 (VeVe) = P2</td>
<td>0    0    1    8</td>
<td>21</td>
<td>0:1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1 (VEDA x 155)</td>
<td>42   5    2    1</td>
<td>50</td>
<td>1:0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F2 (VEDA x 155)</td>
<td>152  30   8    15</td>
<td>260</td>
<td>3:1</td>
<td>0.513</td>
<td>0.474</td>
</tr>
<tr>
<td>BC1P1</td>
<td>41   5    3    1</td>
<td>50</td>
<td>1:0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BC1P2</td>
<td>118  9    7    50</td>
<td>280</td>
<td>1:1</td>
<td>0.514</td>
<td>0.473</td>
</tr>
<tr>
<td>Glamour (veve)</td>
<td>0    0    1    9</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ratings: 0 = no symptoms, 1 = chlorosis and wilting of cotyledons, 2 = chlorosis and wilting of first true leaves, 3 = symptoms on lower 50% of the foliage, 4 = symptoms on over 50% of the foliage. Plants with rating 0, 1 and 2 considered resistant.

[0087] The response of VEDA line, F1 and BC1P1 to inoculation with isolate TSA-S5 lead to the conclusion, that the factor controlling resistance to race 2 in VEDA line is dominantly inherited.

[0088] This conclusion is supported by the clear cut differences between the resistant and the susceptible segregants in F2 population, resistant plants showing VEDA line level of resistance and susceptible plants severely damaged like line 155. The segregation ratio fits 3 resistant plants: 1 susceptible plant ($\chi$ (3:1) = 0.513, not significant at the 95% confidence level). These results suggest a major gene is involved with resistance to race 2 in VEDA line.

[0089] The reactions of the above mentioned plant materials, inoculated with isolate Vd 9701 were very similar to those obtained with isolate TSA-S5. The results suggest that the dominant gene conferring resistance to isolate TSA-S5 in VEDA line can control also the virulent strain Vd 9701.

[0090] In all of the resistant lines, the plants at two months are essentially symptomless. As noted above, the absence of symptoms at two months level has been adopted as the indicator for high level of resistance to race 2. Figures 4 and 5 are provided to show the levels of resistance. In Figure 4, susceptible control (right) and VEDA line (left) plants are shown side by side at two months following inoculum. The lower 25% to 30% of the susceptible control shows chlorosis and substantial wilting.

[0091] In Figure 5, similar plants are shown at 4 months, with the entire susceptible plant showing chlorosis, wilting and diminished vigor.

[0092] Tomato fruits of the F1 plants are about 5 cm or greater in diameter.
DEPOSIT INFORMATION

[0093] A deposit of the proprietary inbred VEDA line disclosed above has been made with NCIMB Ltd, 23 St. Machar Drive, Aberdeen AB24 3RY. The date of each of these deposits was 16th June 2005. The NCIMB accession numbers for inbred lines NCIMB 41327.

[0094] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the invention, as limited only by the scope of the appended Claims.
CLAIMS

What is claimed is:

1. A cultivated type tomato plant having a high level *Verticillium* race 2 resistance.


3. The tomato plant of Claim 2 further having resistance to *Verticillium* race 1.

4. The tomato plant of Claim 3 comprising the *Ve* gene.

5. The tomato plant of Claim 1, wherein said plant is homozygous for *Verticillium* race 2 resistance.

6. The tomato plant of Claim 1 wherein the high level *Verticillium* race 2 resistance is dominantly inherited.

7. The tomato plant of Claim 1 wherein the high level *Verticillium* race 2 resistance is derived from a tomato plant of the species *Lycopersicon chilense* Dun.


9. Seed from the plant of Claim 1.


11. Ovule from the plant of Claim 1.

12. Vegetative tissue from the plant of Claim 1.

13. A tomato plant regenerated from tissue of Claim 12.

14. A method for producing hybrid tomato seed comprising crossing an inbred tomato plant of Claim 5 with a second tomato plant and harvesting resultant hybrid tomato seed.

15. A hybrid tomato plant produced by growing the resultant hybrid tomato seed of Claim 14.

16. A method for producing the tomato plant of Claim 1 comprising:

a. crossing a female cultivated tomato plant with a male tomato plant of the subgenus *Eriopersicon* having race 2 resistance.

17. The method of Claim 16 wherein the male tomato plant is *Lycopersicon chilense* Dun.

18. The method of Claim 17 wherein the female cultivated tomato plant is *Lycopersicon esculentum* Mill.

19. The tomato plant of Claim 1, wherein the plant produces a fruit having a diameter of at least about 5 cm.

20. A tomato fruit harvested from a plant according to Claim 1.

21. Tomato seed derived from the tomato fruit of Claim 20.

22. A plurality of tomato plants according to Claim 1 grown in a field.
23. A plant derived from the plant of Claim 23 having heritable high level
Verticillium race 2 resistance.
24. The plant of Claim 24 wherein said plant is a direct genetic descendent.
25. A tomato plant having the high level Verticillium race 2 resistance
characteristic of a plant produced from seed deposited as Accession No. NCIMB 41327.
26. The tomato plant of Claim 25 descended from a plant first produced as seed
deposited as Accession No. NCIMB 41327.
27. The tomato plant of Claim 26 having resistance to Verticillium race 1.
31. Vegetative tissue from the plant of Claim 26.
32. A tomato plant regenerated from tissue of Claim 31.
33. A method for producing hybrid tomato seed comprising crossing an inbred
tomato plant of Claim 26 with a second tomato plant and harvesting resultant hybrid tomato
seed.
34. A hybrid tomato plant produced by growing the resultant hybrid tomato seed
of Claim 33.